

4.2.1 Vertebral Fusion Studies

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In Vivo Results

Stryker Study 07-003: Single-Level Posterior-Lateral Lumbar Vertebral Transverse Process Fusion in Baboons with BMP-7 (OP-1 Putty) and Instrumentation over 4 Months

The objective of this study was to characterize the dose response of the concentration of OP-1 in OP-1 Putty using an instrumented model of single level posterior-lateral vertebral transverse fusion in baboons. Iliac crest autograft was used as a positive control. Concentrations of OP-1 ranging between 0.33 mg/mL and 4 mg/mL (4-48 mg total) were used in the animals. Fusion was evaluated at 3 and 4 months after surgery.

Posterolateral lumbar vertebral fusion surgery included internal fixation and implantation of 12 mL of OP-1 Putty (6 mL per side) with varying dose concentrations of OP-1, or iliac crest bone graft (Autograft), at a single level (between lumbar vertebrae 3 [L3] and L4). Six groups of 4 adult male baboons were implanted with autograft or 0, 0.33, 1, 2, or 4 mg/mL of OP-1 in carrier (a total of 0, 4, 12, 24, or 48 mg of OP-1). Efficacy was assessed by evaluation of computed tomography (CT) images and biomechanical data. Serum and tissue samples as well as plain film radiographs of the fusion site were obtained for eventual analysis. General health of the animals was assessed by evaluation of clinical pathology (hematology, serum chemistry), for product safety by clinical signs, body weights, organ weights, necropsy, and microscopic analysis of selected tissues (subcutaneous implant site, brain, liver, kidneys, spleen, thymus, lymph nodes, intestines, pancreas).

Complete fusion was observed in all animals at OP-1 concentrations ≥ 1 mg/mL and in the autograft treated animals by 3 months. In the 0.33 mg/mL group, 50 and 75% of animals were fused at 3 and 4 months, respectively. Carrier alone was not associated with fusion. In a biomechanical assessment of lateral bending, the carrier control displayed more motion at the fusion site compared with the other treatments (all OP-1 doses and autograft). Biomechanical assessment of the fusion site did not differentiate among treatment groups in axial rotation or flexion/extension.

Refer to P060021/A011 Appendix 1a-2 for an interim study report.

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Stryker Study 07-012: Assessment of potential toxicity and toxicokinetics of OP-1 Putty in male and female New Zealand White Rabbits via a postero-lateral vertebral fusion model

The main objective of this study was to assess the potential toxicity of OP-1 Putty in a clinically relevant surgical model, posterolateral vertebral transverse process fusion (PLF), in male and female New Zealand White (NZW) rabbits (Refer to P060021/A011, Section 4.4, page 52). However, a secondary purpose was to assess efficacy of the fusion. A toxicokinetic (TK) profile of OP-1 released from implanted OP-1 Putty was also performed (Refer to P060021/A011, Section 3.2.2, page 39). Five treatment groups (N = 3/gender/group) were used, including a sham surgery (untreated) control group (Group 1) and four treatment groups. Animals in Groups 1 through 4 had surgery. Group 1 (sham) had surgery alone (no OP-1) and no placement of material. Groups 2 through 4 had 3 mL of OP-1 Putty implanted; 1.5 mL was placed on each side of the vertebrae between transverse processes L4 and L5. The concentrations of OP-1 in Groups 2, 3 and 4 were 1, 2 and 4 mg/mL, respectively. Animals, assigned to Group 5, were injected IV with 1 mg/kg of 6 mg/mL OP-1 on Day 0; these animals served as positive controls for a serum OP-1 ELISA only. All animals were monitored for clinical observations, weekly body weight, and daily feed consumption. TK serum samples were also collected. Serum samples for eventual measurement of antibodies directed against OP-1 were collected before surgery and on Day 35. Clinical pathology parameters (hematology, serum chemistry, coagulation, and urinalysis) were monitored pre-dose and before euthanasia. On Day 35, all animals were euthanized and selected tissues/organs were collected for possible evaluation.

Compared with the control (Group 1), growth parameters (body weight and body weight changes), clinical pathology (hematology, chemistry, coagulation, and urinalysis), and organ weights (absolute and relative) were not affected by administration of OP-1 Putty or OP-1 IV at any dose level. As expected, all animals surgically implanted with OP-1 Putty (Groups 2 through 4) displayed bone formation based on radiographs, macroscopic assessments of transverse process fusion, and microscopic examination of the fusion sites. No ectopic bone was observed away from the fusion sites or the directly adjacent surgical sites.

Toxicokinetic and Toxicity data are described in detail in (Refer to P060021/A011 Sections 3.2.2 and 4.4 pages 39 and 52, respectively). In short, very low serum levels of OP-1 were detected through day 35. No adverse findings were observed in any of the OP-1 treatment groups.

In conclusion, animals treated with OP-1 Putty had bony fusion of the vertebral transverse processes. Administration of OP-1, either in the OP-1 Putty or by the IV route was not

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associated with adverse findings. The TK analysis was consistent with low level release of OP-1 from the implant site over time.

Refer to P060021/A011 Appendix 1a-1 for an interim study report.

Stryker Biotech Study 99-032: Osteogenic Protein-1 (OP-1®) Putty versus Autograft for Posterolateral Spinal Arthrodesis. An *In Vivo* Time-Course Study Using a Canine Model

The objective of this study was to compare the mechanisms of posterolateral osseointegration induced by treatments of OP-1® Putty (0.4 to 1.0 mg/mL OP-1) alone, OP-1® Putty in combination with iliac crest autograft, and autograft alone utilizing lumbar spinal uninstrumented arthrodesis of two noncontiguous levels. The time course of fusion maturation among these three experimental treatments was assessed by surgical examination, radiography, biomechanical testing, and histopathology.

All animals survived the surgery and postoperative time period without incidence of vascular, neurologic, or infectious complications. Radiographic differences in fusion maturation among the treatment groups were evident at the 4 week time point and were sustained throughout the 24 week interval. OP-1 treatments demonstrated an accelerated rate of radiographic fusion by the 4 week time point (66% OP-1® Putty, 88% autograft/OP-1® Putty, 22% autograft) compared with autograft alone and was sustained throughout the observation period. Nondestructive biomechanical testing of the 4 week postoperative arthrodeses displayed no significant differences in peak range of motion among the three treatment groups under any loading modality. By 12 weeks, the range of motion for the autograft/OP-1® Putty treatment was lower compared with autograft alone. The lower range of motion in the OP-1 treatment groups is indicative of increased fusion, improved healing and remodeling of newly formed bone.

Histologic evaluation of the autograft/OP-1® Putty and OP-1® Putty alone treatments at 4 weeks revealed that the intertransverse trabecular architecture was generally complete and composed of a dense network of woven, spindle-shaped trabecular bone. In contrast, corticocancellous bone fragments were found within the intertransverse space in the autograft group, with a connective tissue interface primarily consisting of Type II collagen bundles and cartilage. By 8 weeks, the corticocancellous autograft chips appear further osseointegrated into the arthrodesis sites and the presence of connective tissues, collagen, and cartilage were evident throughout. In the autograft/OP-1® Putty and OP-1® Putty groups at 8 weeks, the fusion mass was more organized and thicker than the 4 week specimens, with an extensive Type II collagen network throughout. Most of the trabeculae were composed of woven bone with thick osteoid seams. By 12 weeks, the autograft treatments, in many cases, demonstrated complete intertransverse fusion bridges

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and contained more lamellar than woven trabeculae. Those sites in the OP-1[®] Putty groups demonstrated fusion mass trabeculae that were well organized and primarily composed of lamellar bone. By 24 weeks, confluent bridges of mature lamellar trabecular bone extending between and adjoining the operative level transverse processes were evident in the OP-1 treated groups.

The data show that OP-1[®] Putty treated specimens had higher quality bone than autograft alone treated specimens in this canine model of uninstrumented lumbar spine fusion. The nondestructive mechanical test results show that in dogs, OP-1[®] Putty alone or as an adjunct to autograft produced fusion with superior integrity compared to autograft alone.

See P060021; Appendix 2, page 645 for complete study report.

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Stryker Biotech Study 00-029: Efficacy of OP-1[®] Putty in a Multilevel Spinal Fusion in Sheep

The objective of this study was to evaluate the capacity of OP-1 (1.0 mg/mL OP-1) to induce healing in a challenging multilevel fusion model. The study was a contiguous uninstrumented 3 level fusion attempt in the sheep lumbosacral region (L5 to S1).

All animals survived for the duration of the 6 month study, and no complications were encountered. Although high rates of nonunion were observed in all groups, 44% of the autograft-treated animals and 56% of OP-1[®] Putty animals displayed fusion between L5 and S1. The carrier group displayed little or no fusion in any of the animals. OP-1[®] Putty and autograft outperformed carrier alone for each measured parameter. Some differences between OP-1-treated and autograft groups were observed. Specimens from OP-1-treated animals yielded more continuous, thicker fusion sites than autograft-treated animals. However, results from the biomechanical and histology testing were inconsistent. OP-1 demonstrated better fusion rates by biomechanical evaluation at levels L6-L7, L7-S1, and L5-6-7-S1, but autograft was more effective at L5-L6. Histologic evaluation yielded similar results.

In conclusion, no consistent differences were observed between groups treated with OP-1[®] Putty and iliac crest autograft. OP-1[®] Putty and autograft significantly outperformed carrier-alone treatment for all parameters evaluated. Results from this sheep spinal fusion study support the use of OP-1[®] Putty as an alternative to autograft in this sheep spinal fusion model.

See P060021; Appendix 2, page 892 for the complete study report.

Stryker Biotech Study 02-002: Comparison of Posterolateral Lumbar Fusion Rates of Grafton[®] Putty and OP-1[®] Putty in Athymic Rat Model

The objective of this study was to compare the osteoinductive properties of OP-1[®] Putty and Grafton[®] DBM Putty (Demineralized Bone Matrix, Osteotech, Inc.), a commercially available osteoinductive product in a rat model of posterolateral fusion. Athymic rats were used to alleviate potential host response to the xenograft in Grafton[®] DBM Putty. Similar volumes (2.0 mL/kg) of either OP-1[®] Putty (1 mg/mL OP-1) or Grafton[®] Putty were applied to a single-level uninstrumented intertransverse process fusion and the rats were followed for either 3 or 6 weeks.

The OP-1[®] Putty group was fused more quickly and had a significantly higher fusion rate than the Grafton[®] Putty group at 3 and 6 weeks as assessed by manual palpation, histology, and radiography of the fused lumbar segment. At 3 weeks, all of the animals were already fused in the OP-1[®] Putty group, while the only 16% of the rats in the Grafton[®] Putty group were fused. At 6 weeks, 100% of animals in the OP-1 group were fused while only 39% of the rats receiving

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Grafton[®] Putty were fused. All OP-1 specimens demonstrated fusion at one or two adjacent levels. In conclusion, the use of OP-1[®] Putty in this rat spinal fusion model was significantly more efficacious than a comparable amount of DBM, emphasizing its osteoinductive properties and importance to the bone healing process.

See P060021; Appendix 2, page 930 for complete study report.

Stryker Biotech Study 02-017: A Rabbit Pseudarthrosis Model and Evaluation of the Role of OP-1 to Overcome Pseudarthrosis

The objective of this study was to establish a model of pseudarthrosis repair and to evaluate the ability of OP-1[®] Putty to induce fusion under these conditions. Uninstrumented posterolateral lumbar fusions were performed with autograft (2 mL per level) in nicotine-exposed (osmotic pump at 4.5 µg/kg/minute for 5 weeks) New Zealand white rabbits. Animals that developed a pseudarthrosis (nonunion), as assessed by palpation, were either left untreated or regrafted with autograft (2.0 mL per level) or OP-1[®] Putty (1 mg/mL OP-1). Nicotine pumps were reapplied at the time of second surgery for an additional 5 weeks. Total duration of the study was 10 weeks.

By manual palpation at 10 weeks, 10% of the untreated pseudarthrosis animals fused, 42% of pseudarthrosis animals receiving autograft fused, and 82% of pseudarthrosis animals receiving OP-1[®] Putty fused. The rate of fusion in rabbits receiving OP-1 was significantly higher than in animals receiving autograft only or those receiving no treatment. The rate of fusion in rabbits receiving autograft was not significantly higher than the rate of fusion among rabbits receiving no graft. Based on the results from this model, OP-1[®] Putty appears to have more potential than autograft for inducing spinal fusion in a poor healing environment.

See P060021; Appendix 2, page 936 for complete study report.

Stryker Biotech Study 02-005: The Effects of OP-1[®] Putty in Instrumented vs. Uninstrumented Lumbar Fusion in Sheep

The objective of this study was to assess the effects of instrumentation when combined with OP-1[®] Putty (1 mg/mL OP-1) in a posterolateral spine fusion model. Sheep were treated either with OP-1[®] Putty alone or OP-1[®] Putty with instrumentation. The duration of the study was 12 weeks with an interim assessment at 6 weeks.

The differences between the treatment groups were somewhat variable. Based on X-ray scores, the uninstrumented group had significantly more fusion at 6 weeks than did the instrumented animals; no differences were observed between groups based on CT evaluation. At 12 weeks, the uninstrumented group displayed significantly improved fusion compared with the

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instrumented group; however, this observation was based on CT scans. No differences were seen between groups at 12 weeks with X-ray analysis. The uninstrumented group had significantly greater amounts of bone present within the callus area at 12 weeks, significantly fewer discontinuities, and more continuous bone than the instrumented group.

In conclusion, the results of this study showed that OP-1[®] Putty in an unstable environment yielded equivalent or superior fusion as compared to a stable environment with instrumentation. The use of instrumentation combined with OP-1 treatment did not prove to be beneficial in sheep undergoing posterolateral lumbar fusion.

See P060021; Appendix 2, page 998 for complete study report.

Stryker Biotech Study 97-038: A Study Evaluating the Effects of the Osteogenic Protein Device on Neurological Tissue in a Canine Laminectomy and Posterolateral Spinal Fusion Model

The objective of this study was primarily to evaluate the efficacy of OP-1[®] Putty (0.1 mg/mL OP-1) and OP-1[®] Implant (1.0 mg/mL OP-1) in an uninstrumented posterolateral spinal fusion model. Additionally, the effects of OP-1[®] Putty administered in the subdural space were examined to evaluate potential consequences to the spinal cord and surrounding structures.

Mixed breed dogs were used in the study. The midline fascia was opened and the paraspinal muscles were dissected to the transverse processes and a laminectomy was performed. Bone fragments were reserved. The dura was identified and opened longitudinally at the midline. The arachnoid membrane was also opened. OP-1[®] Putty or OP-1[®] Implant was reconstituted with 2.5 mL sterile saline. A small amount (approximately 0.25 mL) was placed in the subarachnoid space and pushed laterally under the arachnoid membrane, after which the dura was closed. The dura was not opened and no subdural mass was implanted under the dura in sham animals. Viewed in retrospect, this was a significant design flaw in the protocol. Transverse processes were debrided for posterolateral fusion. In the control animals, bone fragments were laid down over denuded bone. In the OP-1 groups, the remaining OP-1[®] Putty or OP-1[®] Implant was equally divided and placed over denuded bone, followed by the bone fragments. Animals were euthanized 16 weeks after surgery.

Four (4) animals were excluded from fusion analysis due to inadequate exposure of transverse processes. Ninety one percent (91%) of the animals in the OP-1[®] Putty group were fused as determined by palpation. Eighty percent (80%) of the animals also fused in the OP-1[®] Implant group. Twenty-five percent (25%) of the animals in the control group fused. Most animals in each OP-1 group demonstrated calcification at the site of the laminectomy defect. OP-1-treated

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dogs developed new bone in the subarachnoid space; however, no clinical or pathological features of neurotoxicity were noted in any of these animals. Thirty-three percent (33%) of the animals in the OP-1[®] Implant group and 25% of the animals in the OP-1[®] Putty group exhibited a stenosis of the cord in association with implant at the time the dural tube and cord were harvested from the spine. Spinal stenosis was measured as the thickness of the operated canal relative to an adjacent unoperated segment. The mean amount of narrowing noted was 1.7 mm per side in the OP-1[®] Implant group, 1.1 mm per side in the OP-1[®] Putty group, and 0.3 mm per side in the control group. The differences between the OP-1[®] Implant and OP-1[®] Putty groups were significantly different from control; however, the difference between the OP-1 groups was not significant. Because a control mass was not implanted in the sham animals, it is impossible to know whether the transient effects noted were related to the effects of OP-1 per se, or due to the compression of the spinal cord caused by mass implantation. Clearly, even transient compression of the spinal cord can have significant physiologic consequences (Delamarter, Sherman et al. J Bone Joint Surg Am 77(7): 1042-1049; 1995)

In conclusion, OP-1[®] Putty and OP-1[®] Implant effectively fused the lumbar spine in this canine model. Moreover, although new bone formed in the subdural space after placement of OP-1 under the dural membrane, the new bone appeared to have few, if any, negative consequences for the neurologic parameters evaluated. Moreover, due to a flaw in the experimental protocol, it is not possible to determine whether the effects of sub-dural implantation were caused by OP-1 or to non-specific effects of mass implantation under the dural membrane.

See P060021; Appendix 2, page 1025 for complete study report.